AMENDMENTS TO THE SPECIFICATION

On page 14, please delete the paragraph beginning on line 29 and ending on page 15, line 8, and replace with the following paragraph:

It is also not intended that the present invention be limited by the ribosome binding site. In one embodiment, the present invention contemplates primers comprising the *Kozak sequence*, a string of non-random nucleotides (consensus sequence 5'-GCCA/GCCATGG-3' [SEQ ID NO:1]) which are present before the translation initiating *first* ATG in majority of the mRNAs which are transcribed and translated in an eukarytic cells. See M. Kozak, *Cell* 44:283-292 (1986). In another embodiment, the present invention contemplates a primer comprising the the prokaryotic mRNA ribosome binding site, which usually contains part or all of a polypurine domain UAAGGAGGU [SEQ ID NO:2] known as the Shine-Dalgarno (SD) sequence found just 5' to the translation initiation codon: mRNA 5'-UAAGGAGGU - N_{5-10} - AUG [SEQ ID NO:3].

On page 19, please delete the paragraphs beginning on line 2 and ending on line 4, and replace with the following paragraphs:

Figure 1 shows the structure of (A) an amino acid and (B) a peptide [SEQ ID NO:15].

Figure 2 [SEQ ID NOS:16 to 18] provides a description of the molecular steps that occur during protein synthesis in a cellular or cell-free system.

On page 36, please delete the paragraph beginning on line 29 and ending on page 37, line 10, and replace with the following paragraph:

One class of fluorescent markers contemplated by the present invention is the class of small peptides that can specifically bind to molecules which, upon binding, are detectable. One example of this approach is the peptide having the sequence of WEAAAREACCRECCARA [SEQ ID NO:4]. This sequence (which contains four cysteine residues) allows the peptide to specifically bind the non-fluorescent dye molecule 4', 5'-bis(1,3,2-dithioarsolan-2-yl) fluorescein (FLASH, which stands for fluorescein arsenic helix binder). This dye has the interesting property that, upon binding, it becomes fluorescent. In other words, fluorescence is observed only when this specific peptide sequence is present in

the nascent protein. So by putting the peptide sequence at the N- or C-terminal, one can easily monitor the amount of protein synthesized. This peptide sequence can be introduced by designing the nucleic acid primers such that they carry a region encoding the peptide sequence.

On page 45, please delete the paragraph beginning on line 1 and ending on line 15, and replace with the following paragraph:

In one example, a detectable marker comprising a non-native amino acid or amino acid derivative is incorporated into the nascent protein during its translation at the amino terminal (N-terminal end) using a misaminoacylate initiator tRNA which only recognizes the AUG start codon signaling the initiation of protein synthesis. One example of a detectable marker is the highly fluorescent compound BODIPY FL. The marker might also be photocleavable such as photocleavable coumarin or photocleavable biotin. The nascent protein is then separated from the cell-free or cellular translation system by using a coupling agent which binds to an affinity marker located adjacent to the N-terminal of the protein. One such affinity marker is a specific protein sequence known as an epitope. An epitope has the property that it selectively interacts with molecules and/or materials containing acceptor groups. There are many epitope sequences reported in the literature including HisX6 (HHHHHH) [SEQ ID NO:5] described by ClonTech and C-myc (-EQKLISEEDL) [SEQ ID NO:6] described by Roche-BM, Flag (DYKDDDDK) [SEQ ID NO:7] described by Stratagene), SteptTag (WSHPQFEK) [SEQ ID NO:8] described by Sigma-Genosys and HA Tag (YPYDVPDYA) [SEQ ID NO:9] described by Roche-BM.

On page 48, please delete the paragraph beginning on line 13 and ending on line 25, and replace with the following paragraph:

For optimal effectiveness, the N-terminal marker and affinity marker should be placed as close as possible to the N-terminal end of the protein. For example, if an N-terminal marker is incorporated using a misaminoacylated initiator, it will be located at the N-terminal amino acid. In this case, the affinity marker should be located immediately adjacent to the N-terminal marker. Thus, if a BODIPY marker which consists of a BODIPY conjugated to methionine is incorporated by a misaminoacylated initiator tRNA, it should be followed by

an epitope sequence such as SteptTag (WSHPQFEK) [SEQ ID NO:8] so that the entire N-terminal sequence will be BODIPY-MWSPQFEK [SEQ ID NO:10]. However, for specific cases it may be advantageous to add intervening amino acids between the BODIPY-M and the epitope sequence in order to avoid interaction between the N-terminal marker and the affinity marker or the coupling agent which binds the affinity marker. Such interactions will vary depending on the nature of the N-terminal marker, affinity marker and coupling agent.

On page 54, please delete the paragraph beginning on line 9 and ending on line 24, and replace with the following paragraph:

One type of interaction between the markers that is advantageously used causes a fluorescence resonant energy transfer which occurs when the two markers are within a distance of between about 1 angstrom (A) to about 50 A, and preferably less than about 10 A. In this case, excitation of one marker with electromagnetic radiation causes the second marker to emit electromagnetic radiation of a different wavelength which is detectable. This could be accomplished, for example, by incorporating a fluorescent marker at the N-terminal end of the protein using the E. coli initiator $tRNA^{fmet}$. An epitope is then incorporated near the N-terminal end such as the SteptTag (WSHPQFEK) [SEQ ID NO:8] described by Sigma-Genosys. Streptavidin is then conjugated using known methods with a second fluorescent marker which is chosen to efficiently undergo fluorescent energy transfer with marker 1. The efficiency of this process can be determined by calculating the a Forster energy transfer radius which depends on the spectral properties of the two markers. The marker-streptavidin complex is then introduced into the translation mixture. Only when nascent protein is produced does fluorescent energy transfer between the first and second marker occur due to the specific interaction of the nascent protein StreptTag epitope with the streptavidin.

On page 101, please delete the paragraph beginning on line 24 and ending on page 102, line 3, and replace with the following paragraph:

To demonstrate the changes in the electrophoretic mobility of fluorescently labeled nascent protein on the SDS-gels either due to proteolysis or oligomerization in presence of membranes, we have use plasmid DNA of α-hemolysin (α-HL) which codes 32 kDa protein bearing a sequence His-His-His-His-His-His (His-6) [SEQ ID NO:5] at its C-terminal. *In*

vitro translation of α-HL gene was carried out using *E. coli* T7 circular transcription/translation system (Promega Corp., Wisconsin-Madison, WI) in presence of BODIPY-FL-methionyl-tRNA^{fmet} (AmberGen, Inc.) This experiment involved 1) preparation of the tRNA-marker for introduction of the N-terminus marker during translation, 2) translation, 3) purification, 4) protease treatment or 5) oligomerization.

On page 113, please delete the paragraph beginning on line 2 and ending on line 19, and replace with the following paragraph:

Plasmid DNA for α-hemolysin, pT7-WT-H6-αHL, was amplified by PCR using following primers. The forward primer (HL-5) was: 5'-GAATTC-TAATACGACTCACTATAGGGTTAACTTTAA**GAAGG**AGATATACATATG**GAACAAAAATTAATCTCGGAAGAGGATTTG***GCAGATTCTGATATTAATATTAAAACC*-3' [SEQ ID NO:11] and the reverse primer (HL-3) was: 5'-AGCTTCATTAATGATGGTGATGG-TGGTGAC 3' [SEQ ID NO:12]. The underlined sequence in forward primer is T7 promoter, the region in bold corresponds to ribosome binding site (Shine-Dalgarno's sequence), the bold and underlined sequences involve the C-myc epitope and nucleotides shown in italics are the complimentary region of α-hemolysin sequence. In the reverse primer, the underlined sequence corresponds to that of HisX6 epitope. The PCR reaction mixture of 100 ul contained 100 ng template DNA, 0.5 uM each primer, 1 mM $MgCl_2$, 50 ul of PCR master mix (Qiagen, CA) and nuclease free water (Sigma Chemicals, St. Louis, MO) water. The PCR was carried out using Hybaid Omni-E thermocyler (Hybaid, Franklin, MA) fitted with hot-lid using following conditions: 95°C for 2 min, followed by 35 cycles consisted of 95°C for 1 min, 61°C for 1 min and 72°C for 2 min and the final extension at 72°C for 7 min. The PCR product was then purified using Qiagen PCR clean-up kit (Qiagen, CA). The purified PCR DNA was used in the translation reaction.

On page 115, please delete the paragraph beginning on line 16 and ending on page 116, line 3, and replace with the following paragraph:

For PCR the following primers were used: forward primer: 5'*GGATCC*TAATACGACTCACTATAG*GGAGACCACCATGGAACAAAAATTAATATCGGAAGAGGATTTGAATGTTTCTCCATACAGGTCACGGGGA*-3' [SEQ ID NO:13] Reverse